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REVIEW

Role of the Arbuscular Mycorrhizal-associated Rhizobacteria in the Soil borne Phytopathogens in Salem Districts

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Role of the Arbuscular Mycorrhizal-associated Rhizobacteria in the Soil borne Phytopathogens in Salem Districts

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Abstract

The mutualistic symbiosis of most land plants with arbuscular mycorrhizal (AM) fungi has been shown to favour mineral and water nutrition and to increase resistance to abiotic and biotic stresses. This review reports the main mechanisms involved in the control of the disease symptoms and of the intraradical proliferation of soil borne phytopathogens by root colonization with AM fungi, with a special emphasis on the role of the rhizobacteria shown to be specifically associated with the AM extra radical network and the mycorrhizosphere (the soil zone with particular characteristics under the influence of the root/AM association). The mycorrhizosphere would constitute an environment conducive to microorganisms antagonistic to pathogen proliferation. Moreover, attempts to identify rhizobacteria from AM structures and/or the mycorrhizosphere often lead to the isolation of organisms showing strong properties of antagonism on various soil borne pathogens. The ability of AM fungi to control soil borne diseases would be strongly related to their capacity to specifically stimulate the establishment of rhizobacteria unfavourable to pathogen development within the mycorrhizosphere before root infection (*Akhthar et al., 2009*).

Keywords: AM-associated bacteria (AM), arbuscular mycorrhizal fungi, bio control, mycorrhizosphere, Paenibacillus, soil borne pathogens.

INTRODUCTION

Various types of microorganisms are present in soil, play vital roles in numerous physiological activities. These dynamic activities are mediated by association of microorganisms participating in saprophytic, pathogenic and symbiotic association with root. Mycorrhizal fungi are ubiquitous, occurring in all natural ecosystems in most climatic zones throughout the world. The mycorrhizal habitat probably evolved as a survival mechanism for both partners in the association, allowing each to survive in environment of low fertility, drought, disease and temperature extremes

where, alone they could not. Early morphological classifications separated mycorrhizas into endomycorrhizal, ectomycorrhizal and ectendomycorrhizal associations based on the relative location of fungi in roots (*Andrade G et al., 1997*). These three types were not enough to describe the diversity of mycorrhizal associations. The diagnostic feature of arbuscular mycorrhizae (AM) is the development of a highly branched arbuscule within root cortical cells. The fungus initially grows between cortical cells, but soon penetrates the host cell wall and grows within the cell. As the fungus grows, the host cell membrane invaginates and envelops the fungus, creating a new compartment where material of high molecular complexity is deposited. The AM fungi are the most intensively studied types of mycorrhizae because they are present in most agricultural and natural ecosystems and play an important role in plant growth, health and productivity*(Singh D.P et al., 1998).*There are only a few genera belonging to Brassicaceae; Chenopodiaceae and Cyperaceae where they are not found due to the presence of glucosinolates and their hydrolysis products isothiocyanates in and around the roots (*Garmendia I et al., 2006*), which are toxic to the growth of fungi. Mycorrhizal colonization begins with the hyphae that arise from soil-borne propagules, large resting spores of the AM fungi or mycorrhizal root fragments. The fungal hyphae penetrate the root between the epidermal cells and form an appressoria in the first cell layers. This stage marks the autotrophic growth of the fungus. The colonizing hyphae pass through the intercellular spaces and then enter the root tissues spreading between and through cells of the cortical root layers. Once the hyphae have reached the inner cortex they grow into the cells and form tree-like structures called 'arbuscules'. These branched hyphae are closely surrounded by the intact host plasmalemma and represent a large surface of cellular contactbetween both symbionts. These facilitate the exchange of metabolites between host and fungus. The arbuscules are probably the main transfer site of mineral nutrients, mainly phosphorus, from the fungus to the plant and of carbon compounds to the fungus. As internal colonization spreads, the extraradical hyphae ramify, and grow along the root surface forming more penetration points. They also grow outwards into the surrounding soil, thus developing an extensive tridimensionalnetwork of mycelium which interfaces with soil particles.*(Singh D.P et al., 1998)*had demonstrated that the length of the external hyphae growing in soilassociated with mycorrhizal roots reaches an average of 1m cm-1 root, but values of up to 10-14 m cm-1 root have also been recorded. These mycelial network can extend several centimeters outwards from the root surface, bridging over the zone of nutrient depletion around roots to absorb low-mobile ions from the bulk soil (mineral nutrients). In return theplant provides the fungus with sugars, amino acids and vitamins essential for its growth.Arbuscular mycorrhizal (AM) fungi found in rhizosphere of several vascular plants and have important roles on sustainable agriculture as well as agricultural ecosystems management. The beneficial effect of indigenous AM fungi on the nutrition of agricultural plants depends on both the abundance and type of fungi present in the soil (*Rudrappa T et al., 2008).* However, the potential for employing AM fungi on a wide scale in agriculture is dependent on the development of crop-growth-

promoting strains of AM, which are superior to native soil population of AM fungi. Therefore, field study is necessary to understand the abundance and type of indigenous AM fungi present in the rhizosphere of the crop. In this view the present study was undertaken to isolate, identify and classify the indigenous AM fungi associated with four experimental plants grown at different localities of Salem.

The occurrence of arbuscular mycorrhizal fungi in South Africa (old name: Endogonaceae) was first reported by Hattingh (1972) when he discovered a honey coloured sessile spore attached to the stalk of an empty mother spore. Arbuscular mycorrhizal (AM) fungi are obligate symbiotic fungi and endosymbionts of a variety of plants within the Angiosperms, Gymnosperms and Pteridophytes (*Harrier L.A et al 2004*). AM fungi have three major components: the root itself which provides carbon in the form of sugars to the fungus, fungal structures within cortical cells of plant root that provide contact between fungus and the plant cytoplasm and the extraradicalhyphae that aid uptake of nutrients and water.

The evolution of AM fungi can be dated back 460 million years ago from fossil records of the Ordovician age. These records suggest that AM fungi may have played a crucial role in colonisation of most terrestrial plants. The taxonomy of AM fungi has been based on morphological and anatomical characteristics of their spores. Previously, there was lack of detailed information on the influence of abiotic factors on indigenous AM fungal species. However, Uhlmann et al., (2004) carried out a comparative study on species diversity of AM fungi in different seasonal\ areas of South Africa and Namibia. Results revealed that geographical distance and rainfall to a lesser extent, influenced species diversity. A consideration of seasonal changes was also suggested by Dames (1991) when AM fungal species responded differently to soil fertility factors such as pH, moisture, percentage carbon, phosphorus and cations. Morton and Redecker (2001) recognized two other families, the Archaeosporaceaeand Paraglomaceae, with two new genera, Archaeospora and Paraglomus. The recent classification proposed by Schwarzott et al., (2001) has been adapted in the present investigation. So far there are 164 species of AM fungi reported from all over the world.

MATERIALS AND METHODS

Study site and Sample collection Occurrence of Arbuscular Mycorrhizal (AM) fungal association was investigated on the four experimental plants such as Corchoruscapsularis L., Crotalaria juncea L., Gossypiumhirsutum L. and Hibiscus cannabinus L. The soil and root samples were collected from the selected seven different locations of Salem Districts .There is a marked diurnal temperature differences: The temperature can be as below as 20.20C in June and high as 34.420C in March. The annual rain fall is 600-850mm. The climatic regions are semi humid or humid. Soil is covered with a hard, compact crust having dark brown colour. Then the samples were brought to the laboratory and the fine roots in each sample were removed, rinsed with tap water and fixed in

formalin acetic alcohol (FAA), for the determination of root colonization. The soil samples were then air dried in the shade at laboratory temperature for spore counting.

Isolation and Quantification of AM fungal spores

The AM fungal spores were separated from the soil by wet sieving and decanting technique (Gerdman and Nicolson, 1963). Fifty gram of rhizospheric soil sample was mixed in 200 ml of distilled water in a large beaker. After 1 hrsthe contents of the beaker were decanted through the sieves which were arranged in a descending order from 400 µm to 25 µm size. The process was repeated for thrice. The procedure was repeated until the upper layer of soil suspension is transparent. The retained material on the sieve was decanted into a beaker with a stream of water and estimation of spores was carried out by modified method of Gaur and Adholeya (1994). A circular filter paper was taken and folded into four equal quadrants. The paper was reopened; two lines were drawn along the two folds to divide the filter paper into four equal quadrants. Vertical lines were drawn on one half of the filter paper so as to divide it into approximately 20 columns about 0.5cm apart. Each column was then numbered and the direction of counting was marked by an arrow. The filter paper was then folded in such a way that the marked portion becomes the receiving surface for the sample during filtration. This filter paper along with sample spores was spread in a bigger Petri dish. The Petri dish was observed under stereo binocular microscope. Two lines were focused in the field and moving the Petri plate, the spores were counted in every space between the two lines and since the lines were numbered and the direction was set, it was easy to keep track of each spore on the filter paper. For the identification of AM fungal spore, single spore or sporocarps were easily picked up from the filter paper with the help of syringe or fine point camel brush and mounted on a glass slide with a drop of polyvinyl lactophenol (PVL) and a cover slip was placed. Subsequently, recovered spores were identified with the help of manual and different taxonomic keys proposed by different workers The following characters are considered for identification sporocarps, spore morphology, size, shape and peridium of spore, sporocarpscolour, wall ornamentation, subtending hyphae and mode of attachment. Some of the important and selected spores were recorded and documented in the form of photographs

Evaluation of AM Fungal colonization

Arbuscular mycorrhizal fungal structure in roots is usually not observed without appropriate staining. Freshly collected root samples should be washed gently and be free from soil particles. Ultrasonic treatment is effective to disperse soil particles closely adhered to roots. Roots were treated with 10 % KOH solution for 30 min to 1-2 hours in a hot bath, depending on thickness of root structure. Treated roots were washed with water and treated with 2 % HCl solution. Acidified root samples are stained with 0.05 % trypan blue (or acid fuchsin) in lactic acid for 10-15 min in a hot bath

or for a few hours without heating. The roots are destained with lactic acid or lacto-glycerol and are now ready for microscopicobservation. The stained roots may be observed first under a dissecting microscope with transmitted illumination and then observed under a compound microscope. Fungal structures are stained and can be easily recognized (Phillips and Hayman, 1970).

Root colonization:

Per cent of AMF colonization was estimated by microscopically examination at 10 X magnification, after clearing of roots in 10% KOH and staining with 0.05% trypan blue in lactophenol according to method described in Phillips and Hyman (1970). The mycorrhizal colonization was determined by using following formula. Per cent of mycorrhizal colonization = Number of root segments colonized X 100

RESULTS

Rhizosperic soil sample from seven different locations were subjected to the recovery of AM fungal spores. The soil samples of different location showed different types of spores. All the recovered spores represent six genera. Namely Acaulospora, Gigaspora, Sclerocystis, Scutellispora, Glomus and Entrophospora. The entire collected rhizosperesample exhibited the presence of varied range of spore population in the soil profile. Highest spore number was observed in the rhizosphere soil of Crotalaria juncea L. collected from Salem. Lowest spore number was noticed in the rhizosperic soil of Gossypiumhirsutum L.

The pH of the soils varied from 6.95 to 7.33 showing slight acidic to moderately alkaline in nature at most of the localities.Total organic carbon content varied 0.567 to 0.816 at various localities . The total AM fungal spore number at different localities varied from 96.8 to 138.6 per 50 g of soil and per cent root colonization was 37. 9 to 58.6. In the present investigation highest spore density was observed in the soil with rich organic matter and slight acidic soil compare to neutral and alkaline soils. There was a wide variation in spore number especially in Glomus species followed by Acaulospora species However, the distribution of Sclerocystis, Gigaspora, Scutellispora and Entrophosporawas very less at all the localities.Maceration and anatomical studies followed by tryphan blue staining revealed different stages with distinct components of AM fungi. Microscopic measurements provided an assessment of the relative abundance of mycelium in root, the density of hyphae within root, the number of entry points, wall thickness and pattern of outer epidermal cells. The coarse aseptatehyphal coils were often seen from initial penetration points (Plate-IX). Most remarkable morphological feature was the variation in the diameter range (3-25µm) among the hyphalfilaments. The thick walled hyphae were almost filled with dense cytoplasmic matrix with oil globules and most of the thick walled hyphae were smooth with a few irregularities in outline. The thin walled hyphae measuring 2-5µm in diameter arises laterally from the main hyphae. It was noted

that the lateral walled hyphae arise directly from the hyphae of the cytoplasmicconnection. Later content of hyphae disappeared with formation of appressoria. It was observed that the fungus grows throughout the cortex, but it does not invade the endodermis or stele. The fungus penetrated from one cell to another forming a new coil. Intracellular hyphae were usually found in the intermediate layers of the cortical parenchyma with a diameter of about 3-6µm. The hyphae run parallel in between the parenchyma to a considerable distance. In some areas, the hyphae exhibited intermittent projections and were at times swollen. Longitudinal hyphal branches in the form of H shape. Arbuscules of various stages of the growth were observed and some were in the state of disintegration. Morphologically arbuscular branches were short, deteriorating and collapsing. Senescent arbuscules were observed in older portion of the mycorrhizal roots as compared to the young mycorrhizal roots. In present study vesicles were less in numbers and were seen intercalary and intracellularly within the infected roots. Vesicles size and shape diffused depending on the anatomy of the root, varying in size (42-48µm), subglobose and large (89-106µm) in diameter. The intercellular vesicles and host walls have distinct contact, whereas the intracellular vesicles usually enclosed in a layer of cytoplasm. The dense granular cytoplasm with full of fat globules were observed in matured vesicles .The outer walls of the vesicles appeared smooth without ornamentation.

REFERENCES

AKHTAR M.S., SIDDIQUI M.A., 2009. Arbuscular mycorrhizal fungi as potential bioprotectants against plant pathogens. In: Mycorrhizae: sustainable agriculture and forestry (Siddiqui Z.A., Akhtar S., Futai K., eds). Ed Springer, The Netherlands. pp. 61-98.

ANDRADE G., MIHARA K.L., LINDERMAN R.G., BETHLENFALVAY G.J., 1997. Bacteria from rhizosphere and hyphosphere soils of different arbuscular-mycorrhizal fungi. Plant Soil 192, 71-79.

ARTURSSON V., JANSSON J.K., 2003. Use of bromodeoxyuridineimmunocapture to identify active bacteria associated with arbuscular mycorrhizal hyphae. Appl Environ Microbiol 69, 6208-6215.

BIANCIOTTO V., GENRE A., JARGEAT P., LUMINI E., BÉCARD G., BONFANTE P., 2004. Vertical transmission of endo bacteria in the arbuscular mycorrhizal fungus Gigasporamargarita through generation of vegetativespores.Appl Environ Microbiol 70, 3600-3608.

BONFANTE P., 2003. Plants, mycorrhizal fungi and endobacteria: a dialog among cells and genomes. Biol Bull 204, 215-220.

CARLSEN S.C.K., UNDERSTRUP A., FOMSGAARD I.S., MORTENSEN A.G., RAVNSKOV S., 2008. Flavonoids in roots of white clover: interaction of arbuscular mycorrhizal fungi and a pathogenic fungus. Plant Soil 302, 33-43.

GARMENDIA I., AGUIRREOLEA J., GOICOECHEA N., 2006.Defence-related enzymes in pepper roots during interactions with arbuscular mycorrhizal fungi and/or Verticilliumdahliae.Biocontrol 51, 293-310.

HARRIER L.A., WATSON C.A., 2004. The potential role of arbuscular mycorrhizal (AM) fungi in the bioprotection of plants against soil-borne pathogens in organic and/or other sustainable farming systems (Special issue: Current research at the Scottish Agricultural College). Pest Manage Sci 60, 149-157.

LARSEN J., BØDKER L., 2001. Interactions between pea root-inhabiting fungi examined using signature fatty acids. New Phytol 149, 487-493.

RUDRAPPA T., BIEDRZYCKI M.L., BAIS H.P., 2008. Causes and consequences of plant-associated biofilms. FEMS MicrobiolEcol 64, 153-166.

SIDDIQUI Z.A., MAHMOOD I., 1998.Effect of a plant growth promoting bacterium, an AM fungus and soil types on the morphometrics and reproduction of Meloidogynejavanica on tomato.Appl Soil Ecol 8, 77-84.

SINGH D.P., SRIVASTAVA J.S., BAHADUR A., SINGH U.P., SINGH S.K., 2004. Arbuscular mycorrhizal fungi induced biochemical changes in pea (Pisumsativum) and their effect on powdery mildew (Erysiphepisi). J Plant Dis Protect 111, 266-272.

SMITH S.E., READ D.J. 2008. Mycorrhizal symbiosis. Academic Press, San Diego, London.

VIGO C., NORMAN J.R., HOOKER J.E., 2000.Biocontrol of the pathogen Phytophthoraparasitica by arbuscular mycorrhizal fungi is a consequence of effects on infection loci. Plant Pathol 49, 509-514.

WHIPPS J.M., 2004. Prospects and limitations for mycorrhizal in biocontrol of root pathogens. Can J Bot 82,1198-1227.

YAO M.K., TWEDDELL R.J., DESILETS H., 2002.Effect of two vesicular-arbuscular mycorrhizal fungi on the growth of micropropagated potato plantlets and on the extent of disease caused by Rhizoctoniasolani.Mycorrhiza 12, 235-242.